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ORIGINAL PAPER

Bioassays for bomb-makers: proof of concept

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Abstract Clandestine bomb-makers are exposed to significant amounts of explosives and allied materials. As with any ingested xenobiotic substance, these compounds are subject to biotransformation. As such, the potential exists that characteristic suites of biomarkers may be produced and deposited in matrices that can be exploited for forensic and investigative purposes. However, before such assays can be developed, foundational data must be gathered regarding the toxicokinetics, fate, and transport of the resulting biomarkers within the body and in matrices such as urine, hair, nails, sweat, feces, and saliva. This report presents an in vitro method for simulation of human metabolic transformations using human liver microsomes and an assay applicable to representative nitro-explosives. Control and metabolized samples of TNT, RDX, HMX, and tetryl were analyzed using high-performance liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) and biomarkers identified for each. The challenges associated with this method arise from solubility issues and limitations imposed by instrumentation, specifically, modes of ionization.

Keywords Explosives · Biomarkers · Metabolism · LC/MS/MS

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Abbreviations

MRM	multiple-reaction monitoring
K_{ow}	octanol/water partition coefficient
4-ADNT	4-amino-2,6-dinitrotoluene
VP	vapor pressure
HLMs	human liver microsomes

Introduction

The energetic materials used in improvised explosives range from diverted military munitions to commercial formulations such as ammonium nitrate and fuel oil (ANFO), urea nitrate, and peroxides. Bombs can also be fabricated using gunpowder, black powder, and combinations leading to vigorous oxidation/reduction reactions. Metal-based- or ammonium-based nitrates, perchlorates, and permanganates are frequently encountered in the latter type of low-explosive formulations.

Significant progress has been made in detection of explosives and precursors in pre- and post-blast residues and in laboratory methods and field devices [1–6]. As of yet, none of these methods target or take advantage of a rich source of evidence, namely biomarkers. During synthesis, fabrication, and construction of IEDs, bomb-makers are inevitably exposed to the energetic materials, precursors, and by-products. It is reasonable to assume that these individuals and those in close proximity to them will ingest potentially significant amounts of these compounds either through acute or chronic exposures.

Bomb-making material ingested into the body is subject to adsorption, distribution, metabolism, and excretion (ADME) just as any xenobiotic compound. Accordingly, biomarkers of exposure may be detected in matrices such as

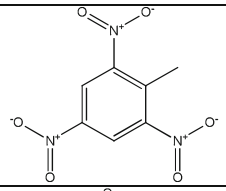
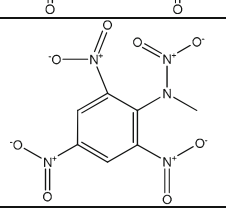
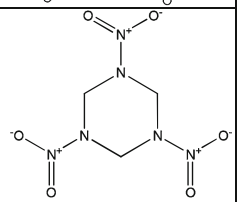
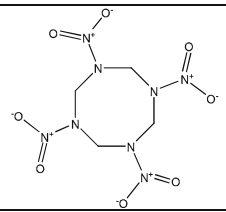
blood, urine, saliva, feces, hair, nails, and sweat. Recent trends in forensic toxicology provide a useful model where significant interest has been directed towards analysis of drugs and metabolites in atypical matrices such as saliva, sweat, and hair [7–11]. There is no reason to think explosives cannot be evaluated in the same way; indeed, some explosives have been used medicinally and share common structural elements with drug molecules.

Before effective assays for explosives biomarkers can be developed, a body of knowledge is needed regarding biodegradation pathways, toxicokinetic factors, and the fate, and transport of these materials within and from the body. Knowing if and where a biomarker may bioconcentrate is also vital. As an example, there have been investigations of explosive deposition on hair [12, 13] used as evidence of exposure, but no reports were found dealing with incorporation of explosives and metabolites *in* the hair. The lipophilic nature of explosives and many of their metabolites suggests that hair would be an ideal matrix for bioconcentration just as it is for drugs and environmental contaminants [14]. Because hair grows at a predictable rate, exposure events could be associated with reasonable confidence to a timeframe of exposure as well as duration and extent of the exposure. Hair collection is non-invasive and is shed naturally, adding to its potential evidentiary and investigative value. Similar arguments can be made for other non-invasively collected biological matrices. However, before these possibilities can be explored, basic data must be collected.

This report describes a proof-of-concept study designed as a first step in filling this gap. In vitro methods typically used for drugs were applied to representative explosives. Assays using tandem LC/MS/MS were used to identify products produced and the results were compared with literature data where available. The explosives targeted are summarized in Table 1.

For these substances, the primary modes of ingestion are expected to be dermal absorption, ingestion, and inhalation. The analytes were selected as representatives of military and commercial explosives and TNT was useful as a control since significant data regarding human exposure is available. Note that TNT has relatively high vapor pressure,

Table 1 Target compounds

Compound	Class	Structure	MW	K_{ow}	LogK_p	Vapor pressure mm Hg (°C)
TNT	Nitrated cyclic aromatic		227.13	1.60	-2.94	8.02×10^{-6} (25)
Tetryl (nitramine)	Nitrated cyclic aromatic		287.14	1.64	-3.01	1.2×10^{-7} (25)
RDX cyclonite	Nitrated cyclic non-aromatic		222.12	0.87	-3.12	4.1×10^{-9} (20)
HMX	Nitrated cyclic non-aromatic		296.15	0.16	-3.76	2.41×10^{-8} (25)

Data for K_{ow} and vapor pressure taken from [34].

Data for K_{ow} and vapor pressure taken from [34].

increasing the likelihood of inhalation exposure. The K_{ow} provides a measure of relative lipophilicity, an important factor in determining dermal penetration. One model used to estimate the skin partition coefficient for volatile organics, expressed as the logK_p value, is [15, 16]:

$$\mathrm{LogK_P} = -2.72 + 0.71\mathrm{LogK_{ow}} - 0.0061\mathrm{MW} \quad (1)$$

where MW is the molecular weight of the compound. While not previously applied to explosives, this quantity is included in Table 1 for comparison purposes. There is ample evidence for dermal absorption of TNT [17], and indications for some small amount of absorbance of RDX [18, 19]. The values of LogK_p suggest that dermal absorption of the other compounds is also likely.

Figure 1 provides an overview of the biotransformation processes of interest. Once ingested, xenobiotic compounds may (1) be excreted unchanged in plasma filtrates such as urine; (2) conjugated with moieties such as glucuronides; or (3) undergo Phase I metabolism with or without conjugation. Products of Phase I metabolism are then subject to these same processes. Depending on various factors (principally, lipophilicity), ingested and transformed compounds may bioconcentrate in fatty tissues or be deposited in hair and other keratinized tissues.

The most common pathway for metabolism of exogenous compounds is via the liver, which contains the cytochrome P450 family of enzymes found on the smooth endoplasmic reticulum. These enzymes are also found in different parts of the body such as the lungs and other tissues. Cytochrome P450 enzymes are monooxygenases in which NADPH-cytochrome P450 reductase, a flavoprotein that donates electrons, acts as a redox partner. The CYP450 superfamily of enzymes catalyzes first-pass or Phase I reactions such as oxidation and hydrolysis [20–22]. Common reactions catalyzed by these enzymes include dehalogenation, epoxidation, hydroxylation of aromatic and aliphatic compounds, dealkylation of nitrogen, oxygen, and sulfur, and the oxidation of phosphorus, sulfur, and nitrogen [23–27]. Although the microsomal enzymes are known for oxidative catalysis, there are instances where these enzymes are active in unusual pathways such as reduction [22, 27, 28]. This is relevant here given that a significant body of literature in human and animal models shows that a key Phase I transition in TNT metabolism is reductive (–NO_2 to –NH_2). Phase II enzymes catalyze conjugation reactions such as glucuronidation [29].

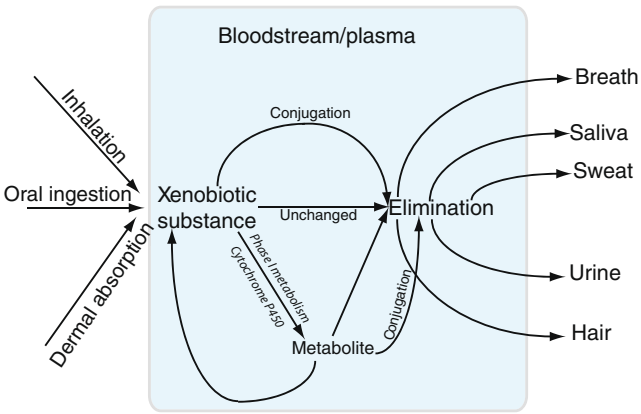


Fig. 1 Overview of ingestion and elimination pathways leading to production of biomarkers of explosives. The present study investigates processes enclosed in the *colored box*

There are several in vitro protocols used to study human hepatic biotransformation reactions and pathway. These include perfused liver, liver slices, hepatocytes and cell lines, and human liver microsomes [30–32]. Each method has advantages and disadvantages and the interested reader is directed to the cited reviews. Human liver microsomes (HLMs) are the most widely used system [30, 31], principally due to low cost, ease of use, and availability. The primary disadvantage of HLMs is that the method is unsuitable for obtaining quantitative data [30]. It is worth noting that none of the current in vitro methods taken alone can completely model human hepatic biotransformation, but in general, they are valuable for identifying primary metabolites [31]. For these reasons, HLMs were selected for use in the present work.

The experimental use of cytochrome P450 applied to explosives has been performed utilizing bacterial, human, and non-human cytochrome P450 enzymes. An in vitro metabolism of TNT was performed utilizing purified rat liver NADPH-cytochrome P450 reductase and cytochrome P450 [33]. The reduced metabolite resulting from NADPH-cytochrome P450 reductase was 4-hydroxylamino-2,6-dinitrotoluene (4HA). With the addition of cytochrome P450, the metabolites, 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-4,6-dinitrotoluene (2-ADNT) were detected. The interpretation of these results suggest that NADPH-cytochrome P450 reductase is responsible for catalyzing the reduction of the nitro group to yield the hydroxylamine. However, cytochrome P450 is the key factor that reduces the hydroxylamine to an amine [33].

Human biomarkers for TNT have been determined in biological fluids [34]. These biomarkers include 4-ADNT bound to hemoglobin (HB) in blood, and 4-ADNT and 2-ADNT found in urine. The median concentration levels of these biomarkers found in Chinese ammunition factory workers were 0.21 μg/mL in urine and 59 ng/g (hemoglobin adduct) in blood. No reports of in vitro metabolism studies of HMX, RDX, or tetryl were located; however, several reports of microbial and environmental degradations exist [35–38]. One report discussed accidental RDX exposure in humans [39] in which high levels of RDX were seen in plasma, but no mention was made of metabolites or conjugates.

For the present work, an in vitro metabolism using human liver microsomes (HLMs) and NADPH was employed and the separation and identification of human metabolites of RDX, HMX, and tetryl were determined using LC/MS/MS. Two layers of control experiments served as method validation. Although intrinsically a qualitative assay, quantitative data was obtained to evaluate extent of metabolism, reproducibility, and to establish LOD and LOQ values for parent explosives and metabolites.

Materials and methods

RDX, HMX, and tetryl were purchased from Cerilliant (Round Rock, TX) as 1,000-ppm solutions in acetonitrile. TNT was purchased in solid form from Chem Service (West Chester, PA). Methanol, ethanol with 0.1% ammonium acetate for LC/MS/MS, H_2O with 0.1% ammonium acetate for LC/MS/MS, acetonitrile, β-nicotinamide adenine dinucleotide phosphate (NADPH), and potassium phosphate monobasic were all purchased from Sigma-Aldrich (St. Louis, MO). Human liver microsomes were purchased from BD Biosciences (San Jose, CA). The viability of the HLMs was verified by control studies using drugs of abuse and previously published procedures typified by [40].

To prepare the RDX, HMX, and tetryl for metabolic studies, the contents of each vial (nominally 1 mL) was transferred to a centrifuge tube and evaporated under a gentle stream of ultrapure nitrogen. Because these explosives are relatively insoluble in water, the concentration of this initial solution could not be calculated. Using the following values for solubilities [41] of ~ 115 mg/L, 60 mg/L, and 140 mg/L for TNT, RDX, HMX, and tetryl, respectively, these initial solutions were estimated to be in the range of 0.1–1.0 mM. Concentrations for control samples were determined using LC/MS/MS with the recognition that this value represents only a reasonable estimate of the concentration of the parent compound initially present in the samples subsequently metabolized. Issues arising related to solubility are discussed in detail in the "Results and discussion" section below.

In vitro metabolism procedure

The in vitro metabolism procedure using HLMs was provided by Dr. Diaa Shakleya from the National Institute on Drug Abuse/Intramural Research facility in Baltimore, MD and adapted based on literature methods applied to drugs, for example, as in [40]. Incubation time must be sufficient for metabolism to occur but not so long as to invite enzyme degradation. In drugs with nitroaromatic functionality such as flurnitrazepam and flutamide, incubation times of 20 [42] and 60 min [43] have been reported. Typical incubation time in drug studies is 30 min [40] and this time was used here; however, a time study was conducted using TNT to confirm this as a reasonable selection. Control and metabolized sample was prepared for each explosive and three replicates of each explosive (except tetryl) were analyzed. The metabolism procedure is illustrated in Fig. 2. NADPH was added only to the samples to be metabolized, the only difference in reagents between metabolized and non-metabolized controls. Acetonitrile extracts were stored at −20 °C until LC/MS/MS analysis.

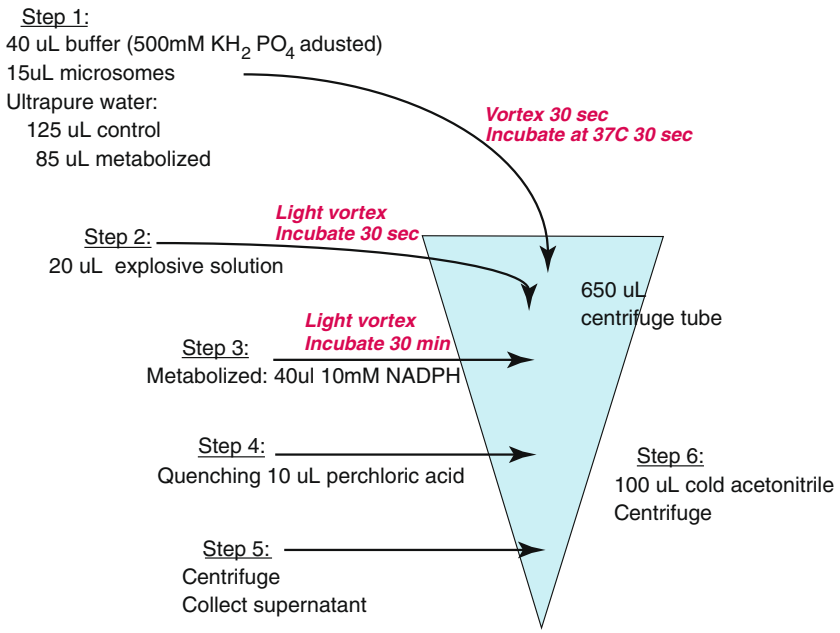


Fig. 2 The in vitro protocol used. The total volume of the solution prior to incubation is always 200 µL

Table 2 MS/MS conditions of TNT, tetryl, RDX, and HMX

Parameter	TNT/tetryl	RDX/HMX
Curtain gas (L/min)	30	30
Collision gas[a]	Medium	Medium
Ion spray voltage	-4500	-4500
Temperature (°C)	600	600
Ion source gas 1 (mL/min)	40	45
Ion source gas 2 (mL/min)	40	45
Declustering potential (V)	-33	-30
Entrance potential (V)	-4.5	-5
Collision energy (V)	-10	-30

[a] As per instrument setting; correlates to a pressure of ~10^{-4} Torr N_2 in the collision cell

Large-volume TNT solution

A significant volume of solution of TNT was required for a time study and for control samples. To prepare this solution, approximately 0.6 g of solid TNT was placed into a screw-cap plastic sample tube to which 50 mL of DI water was added. This was placed on a sample rotator for 36 h to create a saturated solution. At the end of the rotation period, the solution was allowed to settle before aliquots were drawn off.

Quantitation

A seven-point external standard calibration curve was generated for each parent compound at a concentration range of 1.0 µg/L–1.0 mg/L. Each calibration standard was prepared from a 1.0 mg/ml standard of the explosive stored at 4 °C. R^2 values for all curves exceeded 0.99. Previously reported MRM transitions of each explosive were used to determine the ions used for calibration. For quantitation, the MRM transitions were the following: 226 → 46 for TNT, 281 → 46 for RDX, 355 → 46 for HMX, and 241 → 213 for tetryl [44–52]. Limits of detection were determined experimentally for the parent compounds at 8 ng/L for TNT and 500 ng/L for the remaining parent explosives.

HPLC conditions

A Shimadzu Prominence UFLC system was used for the chromatographic separation of metabolites. For TNT analysis, eluent A consisted of ultrapure distilled, organic-free H_2O and eluent B consisted of methanol. Given that RDX and HMX do not readily ionize by ESI, acetate was employed to form an adduct to promote ionization [47]. Thus, eluent A consisted of H_2O with 0.1% ammonium acetate and eluent B was methanol with 0.1% ammonium acetate. Since tetryl was ionized using APCI, the same mobile phase for RDX and HMX was applied. A 20-µL sample was injected onto an Allure C-18 50×2.1 mm, 5-µm particle size column (Restek, Bellefonte, PA, USA). The gradient elution method used for TNT analysis started with 10% B for 3 min and then increased linearly to 100% B to 15 min. Between 15 and 20 min, B was held at 100% and then decreased rapidly to 10% B at 21 min. The run finished at 25 min and the total flow rate was 0.2 ml/min. The isocratic elution applied to RDX, HMX, and tetryl was 58% B with a total flow rate of 1.0 ml/min.

LC/MS/MS conditions

An Applied Biosystems (ABI) 3200 QTrap LC/MS/MS system using electrospray ionization (ESI) was employed for TNT analysis. Atmospheric pressure chemical ionization (APCI) was used for RDX, HMX, and tetryl. In all cases, the negative ion mode was employed and conditions are summarized in Table 2. Two of the potential metabolites of TNT, 2,4-DA-6-NT, and 2,6-DA-4-NT were screened using positive mode. Multiple-reaction monitoring (MRM) transitions of reported TNT human metabolites (Table 3) and animal/microbial metabolites of RDX (Table 4) were used to verify the presence or absence of metabolites.

In addition to metabolites previously reported in the literature, additional possible biotransformation products were identified and screened using Lightsight® software (version 2.0, ABI). The potential metabolites are identified for feasible Phase I transitions (Table 5) based on the parent molecule structure and reported metabolites for similar compounds. The software performs a search for evidence of these transitions based on a comparison of the control and metabolized sample data files. Similar tables were utilized for RDX, HMX, and tetryl. All samples except tetryl were analyzed in triplicate using different lot number explosives including those stored for several weeks and those freshly purchased.

Table 3 MRM transitions of reported human TNT metabolites under various LC/MS/MS conditions

Explosive/metabolite	Parent	MRM
TNT	226	46
2-ADNT (2-amino-dinitrotoluene)	196	136
4-ADNT (4-amino-dinitrotoluene)	196	46
1,3-DNB (1,3-dinitrobenzene)	168	46
1,3,5-Trinitrobenzene (TNB)	213	183
2,4-DNT (2,4-dinitrotoluene)	182	46
2,6-DNT (2,6-dinitrotoluene)	182	46
2,4-DA-6NT (2,4-diamino-6-nitrotoluene)	168	121
2,6-DA-6NT (2,6-diamino-4-nitrotoluene)	168	121

Table 4 MRM transitions of reported animal/microbial RDX metabolites under various LC/MS/MS conditions

Explosive/metabolite	Parent	MRM
RDX	281	46
RDX	297	46
4-nitro-2,4-diazabutamide	133	46, 59, 61
4-nitro-2,4-diazabutanal	118	44, 46, 61
MNX Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine	281	46
TNX Hexahydro-1,3,5-trinitroso1,3,5-triazine	249	113
MEDINA *N*,*N*′-dinitromethanediamine	135	61

Results and discussion

Initial experiments: TNT

Zolpiem (Ambien; aolpidem) was metabolized by human liver microsomes as a positive control for NADPH reductase and CYP450 activity [40]. Results of the assay were in complete agreement with the literature report. The next experimental phase focused on TNT for two reasons: first, because it is readily available as a solid, it was possible to make large volumes of solution essential for replicate analysis. Second, as noted in the introduction, there is a significant body of literature regarding human metabolites available for comparison. Using the procedures previously described, TNT was shown to metabolize to 4-ADNT using the HLMs. This result agrees with the literature regarding human metabolites of TNT [33, 34, 53, 54]. An example chromatogram is shown in Fig. 3.

Once successful metabolism was confirmed, a time study was conducted to ensure that 30 min was a reasonable incubation time. Using aliquots of the TNT solution described above, three replicates were prepared for study with incubation times of 1, 10, 20, 60, and 120 min. For the 30-min trial and controls, five replicate solutions were prepared because these were used in another control experiment described in the next section. To evaluate the progress of metabolism, the appearance of the 4-ADNT metabolite was monitored quantitatively and the ratio of the concentration (ppb) of the parent TNT to the metabolite was calculated. A detectable signal for the metabolite was observed at 20 min, but correlated to a concentration below the LOQ of 1.0 ppb. At 30 min, the ratio was at the lowest (optimal value) of 5.2±4.0 (95% confidence interval). The values at 60 and 120 min were 9.8±2.8 and 17.6±9.5, respectively. It was felt that incubation times beyond this were impractical and were likely to result in enzyme degradation. These results confirmed that 30 min was a reasonable incubation time.

In part to explore possible sources of the relatively large uncertainties seen above, a series of TNT controls were analyzed quantitatively, five replicates each. The first set of five consisted of the aqueous TNT solution; the second, control samples prepared as per Fig. 2 but without addition of NADPH (un-metabolized control); and the third, metabolized samples. All TNT aliquots were drawn from the same stock solution.

As seen in Fig. 4, significant uncertainty arose from the stock solution. If the value of ~115 mg/L for TNT solubility is used as a baseline, the average value of 107.4 ppm determined here is reasonable. However, the range fell between 9 and 191 ppm. The primary reason for this was attributed to the solution; when it was removed from the rotator and allowed to settle, the solution remained slightly turbid with solid residue clearly visible on the surface. Even with careful drawing of aliquots from the clearest central portion of the solution, heterogeneity was obvious. Another interesting and important feature revealed in Fig. 4 is the decrease in TNT concentration observed between the aqueous solution and the controls. Using the *t* test of means, unequal variance, the difference between the means is not statistically significant (α=0.05), but this trend bears exploration once solubility issues are resolved.

Table 5 Possible biotransformations of groups found in target explosives

Biotransformation	Mass shift	Formula
Nitro to amine (×2)	*−60.0*	*–2(–O_2+H_2)*
Nitro to amine	*−30.0*	*–O_2 + H_2*
Loss of H_2O	−18.0	–H_2O
Nitro to nitroso	*−16.0*	*–O*
Demethylation	−14.0	–CH_2
Nitro to hydroxylamine	*−14.0*	*–O + H_2*
Dehydrogenation	−2.0	–H_2
Hydrogenation	+2.0	+H_2
Oxidation	+16.0	+O
Di-oxidation	+32.0	+2O
Tri-oxidation	+48.0	+3O

Transitions in italics are known to occur in TNT

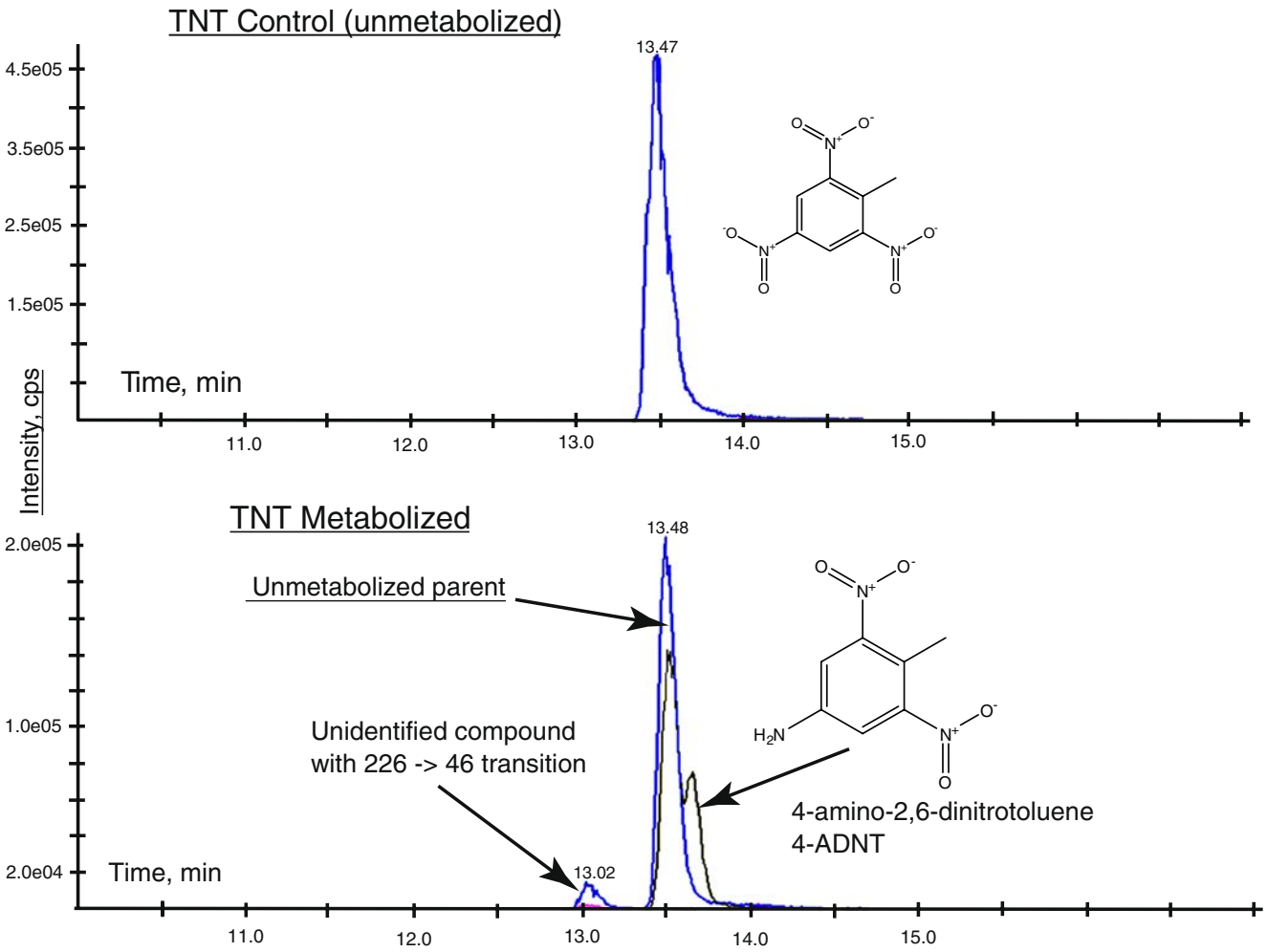


Fig. 3 Overlay of MRM transitions and the resulting chromatogram for metabolized TNT. The unidentified compound was not seen in the blanks or controls and so is likely associated with the metabolism process as either a true metabolite or artifact. The axes have been redrawn for clarity

Other explosives

No metabolic products were observed for the RDX, HMX, and tetryl and no significant or reproducible differences in concentrations between the controls and metabolized samples were seen. A caveat does exist; what is detected is dependent here upon how well the metabolite ionizes under the conditions used. To determine if poorly ionized metabolites were produced but undetected, a set of quantitative data was obtained for the parent compounds in control and metabolized samples (n=3 for each). For TNT, the difference in concentration between control and metabolized solution showed a decrease of ~80% (11.4 ppm and 2.35 ppm, respectively) with concomitant production of confirmed metabolites. There were no significant differences in the parent/metabolized samples from the other explosives and there were no signals observed in the chromatograms in the region above the LOD and below the LOQ.

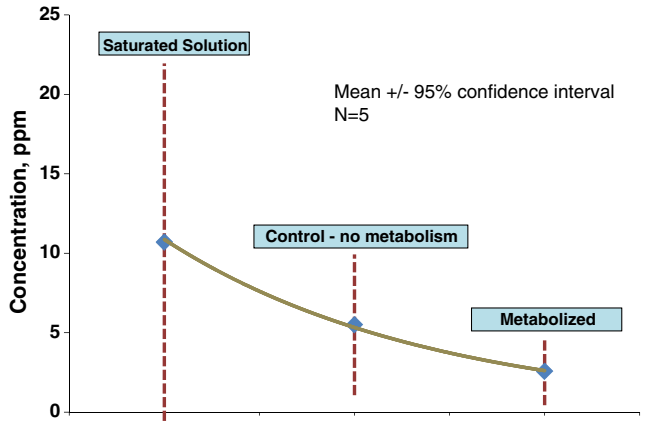


Fig. 4 TNT controls

There are at least three possible explanations for the data produced here showing no biotransformation of RDX, HMX, and tetryl; either none occurred or it was produced but undetected due to poor ionization efficiency or other analytical factors. For example, biotransformation may not

have occurred due to substrate inhibition due to relatively high concentrations of the parent explosive. In terms of ionization, nitrobenzene is a known metabolic product of TNT but it was not detected here, an observation that has been previously reported [55]. Similarly, repeated infusions of TNB, another known TNT metabolite, failed to yield detectable ions. These findings reinforce the need to develop a set of complementary instrumental protocols. Ideal candidates here would be GC/MS or GC/ECD. Although all of the acetonitrile extracts produced here were screened using GC/MS, no peaks were detected; this could indicate either that any analyte present was at trace levels below the detection limit or that no metabolism occurred. This "absence of evidence", as reliable evidence of the absence of significant production of biomarkers, is supported through the control experiments (using the drugs and TNT) and the comparison of control to metabolized concentrations of the parent ions.

Solubility and HLM assays

Metabolism generally occurs in an aqueous environment, modeled in HLM assays by the aqueous-buffered solution. One of the challenges in adapting HLM methods to explosives is the inherently poor water solubility of many of these compounds (Table 1) and the potential incompatible solubilities of products. The small volumes and semi-quantitative nature of the procedure makes it difficult to perform quantitative studies and for this and other reasons, HLM assays are considered to be qualitative [30]. Results here support this cautionary approach.

Although TNT is readily available in solid form, for other explosives, the problem is more significant. Most laboratories will not have access to large amounts of solid RDX, HMX, etc. and commercial solutions are typically used in their place as was the case here. These solutions are usually made in acetonitrile at a nominal concentration of 1.0 mg/mL and are sold in 1-mL vials. Prior to the HLM procedure illustrated in Fig. 2, an aqueous solution of the explosive must be prepared. The contents of the vial are transferred to a centrifuge tube and gently dried under a stream of ultrapure nitrogen, leaving 1.0 mg (nominally) coating the walls of the tube. Some portion of the solid dissolves in the added water, but this amount will depend on intrinsic solubility and temperature. Quantitative data is easily obtained; the problem arises within experimental design. A total volume of 200 μL, not all of which can be recovered from the centrifuge vial, inherently limits the number of replicate experiments that can be conducted. Even with replications (as seen above with TNT), homogeneity of these replicates is in question and so the same "solubility conundrum" arises. Metabolism by HLMs requires an aqueous media; most explosives are not very water soluble. Addressing this challenge is the subject of current research focusing on small amounts of added solvent. This approach has been reported with drugs [56] and has promise here.

Summary and conclusions

TNT was successfully metabolized using HLMs with results in agreement with literature reports. No biotransformations were observed for RDX, HMX, and tetryl. The HLM assay is, by nature, qualitative and two observations are essential to confirm metabolism has occurred and biotransformation products were produced: first, a decrease of concentration of the parent compound and second and most importantly, appearance of a detectable product. If concentration decreases but no product is observed, several factors have to be considered including solubility of products in the mobile phase, chromatography, ionization, concentration, and MRM transitions.

If HLM assays are to migrate successfully to quantitative assays, at least in the present context, if not generally, several sources of uncertainty must be addressed including solubility mismatches and potential variable microsomal activity. Current and future research in this laboratory is focusing on optimizing substrate concentrations, alternative methods of monitoring reaction progress, solubility, implementation of standards and surrogates to improve the ability to track biotransformations for each individual run, and expanding target analyte detection capabilities. These improvements will facilitate mass balance capabilities to better track biotransformation.

In the larger context of developing bioassays for bomb-makers, it is not essential to target trace metabolites. Rather, the goal is to identify the biomarkers produced at the highest concentrations and then to determine in which body compartment or tissue they are likely to be found in the highest concentrations. This study has shown that for TNT, the parent compound along with the metabolite 4ADNT is produced while for RDX, HMX, and tetryl, the best biomarkers of human exposure are the parent compounds. This proof-of-concept work can form the foundation for work with complex and improvised explosive formulations.

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References

1. Gaurav D, Malik AK, Rai PK (2007) Crit Rev Anal Chem 37:227
2. Nambayah M, Quickenden TI (2004) Talanta 63:461
3. Pumera M (2008) Electrophoresis 29:269

4. Schulte-Ladbeck R, Vogel M, Karst U (2006) Anal Bioanal Chem 386:559
5. Tagliaro F, Bortolotti F (2008) Electrophoresis 29:260
6. Wang J (2007) Electroanalysis 19:415
7. de Oliveira CDR, Roehsig M, de Almeida RM et al (2007) Curr Pharm Anal 3:95
8. Gallardo E, Queiroz JA (2008) Biomed Chromatogr 22:795
9. Langman LJ, Kapur BM (2006) Clin Biochem 39:498
10. Mullangi R, Agrawal S, Srinivas NR (2009) Biomed Chromatogr 23:3
11. Palmeri A, Pichini S, Pacifici R et al (2000) Clin Pharmacokinet 38:95
12. Oxley JC, Smith JL, Kirschenbaum LJ et al (2008) J Forensic Sci 53:690
13. Efremenko I, Zach R, Zeiri Y (2007) J Phys Chem C 111:11903
14. Boumba VA, Ziavrou KS, Vougiouklakis T (2006) Int J Toxicol 25:143
15. Fan VS, Savage RE, Buckley TJ (2007) Toxicol Mech Methods 17:295
16. Potts RO, Guy RH (1992) Pharm Res 9:663
17. Reifenrath WG, Kammen HO, Palmer WG et al (2002) Toxicol Appl Pharmacol 182:160
18. Reddy G, Allen NA, Major MA (2008) Toxicol Mech Methods 18:575
19. Reifenrath WG, Kammen HO, Reddy G et al (2008) J Toxicol Env Health Part A 71:486
20. Furge LL, Guengerich FP (2006) Biochem Mol Biol Educ 34:66
21. Guengerich FP (2006) Aaps Journal 8:E101
22. Lee MY, Dordick JS (2006) Curr Opin Biotechnol 17:619
23. Isin EM, Guengerich FP (2008) Anal Bioanal Chem 392:1019
24. Hodgson E, Rose RL (2007) Pharmacol Ther 113:420
25. Wang YH, Li Y, Li YH et al (2006) J Phys Chem B 110:10139
26. Lewis DFV (2003) Pharmacogenomics 4:387
27. Guengerich FP (2001) Curr Drug Metab 2:93
28. Guengerich FP (2001) Chem Res Toxicol 14:611
29. Zamek-Gliszczynski MJ, Hoffmaster KA, Nezasa K et al (2006) Eur J Pharm Sci 27:447
30. Brandon EFA, Raap CD, Meijerman I et al (2003) Toxicol Appl Pharmacol 189:233
31. Dalvie D, Obach RS, Kang P et al (2009) Chem Res Toxicol 22:357
32. Plant N (2004) Drug Discov Today 9:328
33. Leung KH, Yao M, Stearns R et al (1995) Chem-Biol Interact 97:37
34. Sabbioni G, Liu YY, Yan HF et al (2005) Carcinogenesis 26:1272
35. Bhushan B, Halasz A, Thiboutot S et al (2004) Biochem Biophys Res Commun 316:816
36. Gorontzy T, Drzyzga O, Kahl MW et al (1994) Crit Rev Microbiol 20:265
37. Spain JC (1995) Annu Rev Microbiol 49:523
38. Yinon J (1990) Toxicity and metabolism of explosives. CRC, Boca Raton
39. Y. Kucukardali, H. V. Acar, S. Ozkan et al (2003) Accidental oral poisoning caused by RDX (cyclonite): a report of 5 cases GATA Haydarpasa Training Hospital, 81327 Uskudar, Istanbul, Turkey., pages 42-6
40. von Moltke LL, Greenblatt DJ, Granda BW et al (1999) Br J Clin Pharmacol 48:89
41. Hazardous Substances Database (HSDB) (2009) National Library of Medicine—Toxnet: Bethesda. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. Accessed: 18 Feb. 2009
42. Tanaka E, Nakamura T, Terada T et al (2005) Basic Clin Pharmacol Toxicol 96:88
43. Wen B, Coe KJ, Rademacher P et al (2008) Chem Res Toxicol 21:2393
44. Beller HR, Tiemeier K (2002) Environ Sci Technol 36:2060
45. Chen HW, Li M, Jin QH et al (2006) Chin J Anal Chem 34:839
46. Florian J, Gao L, Zhukhovskyy V et al (2007) J Am Soc Mass Spectrom 18:835
47. Gapeev A, Sigman M, Yinon J (2003) Rapid Commun Mass Spectrom 17:943
48. Major MA, Reddy G, Berge MA et al (2007) J Toxicol Environ Health A 70:1191
49. Mathis JA, McCord BR (2005) Rapid Commun Mass Spectrom 19:99
50. Pan XP, Zhang BH, Tian K et al (2006) Rapid Commun Mass Spectrom 20:2222
51. Sigman ME, Armstrong PA, MacInnis JM et al (2005) Anal Chem 77:7434
52. Song LG, Bartmess JE (2009) Rapid Commun Mass Spectrom 23:77
53. Sabbioni G, Rumler R (2007) Biomarkers 12:559
54. Sabbioni G, Sepai O, Norppa H et al (2007) Biomarkers 12:21
55. Schmidt AC, Niehus B, Matysik FM et al (2006) Chromatographia 63:1
56. Rawden HC, Carlile DJ, Tindall A et al (2005) Xenobiotica 35:603